

## SUPPLEMENTARY DATA

### FIGURE LEGENDS

**FIGURE S1.** Real time surface plasmon resonance monitoring of the binding of 20S editosomes to different mitochondrial RNAs. (A) Sensograms of the concentration dependent binding (50mM, 30mM, 15mM, 6mM, 3mM, 2nM top to bottom) of 20S editosomes to: COI mRNA, A6-FE mRNA, Cyb-UE mRNA, Cyb-FE mRNA, A6-UE/gRNA hybrid RNA and gRNA. (B) Corresponding binding curves for the different 20S/RNA interactions. Inserts: Plot of  $k_{on(obs)} = f(\text{conc}_{20S})$  for the calculation of  $k_{on}$  and  $k_{diss}$ . Error bars are relative errors in percent.

**FIGURE S2.** Binding site analysis. (A) Scatchard analysis for the binding of 20S editosomes to surface immobilized RNA molecules to determine the number of RNA binding sites. (B) Summary of the binding site data for all tested RNAs: A6: ATPase subunit 6; Cyb: apocytochrome b; COI: cytochrome oxidase I; gRNA: guide RNA; UE: unedited; FE: fully edited. Error bars are relative errors in percent.

**FIGURE S3.** Substrate RNA competition experiments. Pre-cleaved *in vitro* insertion- and deletion RNA editing assays (7, 30) were performed using pre-annealed radioactively labeled gRNA/pre-mRNA substrate RNAs. Reactions were carried out in the presence of increasing amounts ( $\leq 380$ -fold molar excess) of non-radioactive competitor gRNA/pre-mRNA hybrid RNAs in a reciprocal fashion (U-deletion editing was competed with a U-insertion RNA substrate and *vice versa*). Edited product formation was measured and plotted as a function of the molar excess of competitor RNA. (A) Pre-cleaved U-insertion editing (competed with a U-deletion RNA). (B) Pre-cleaved U-deletion editing (competed with a U-insertion RNA). Half-maximal inhibition for both editing reactions is at  $\leq 10$ nM competitor RNA. Error bars indicate relative errors (%).

**FIGURE S4.** AFM-based RNA contour lengths measurements. Messenger RNA preparations of A6-UE (A), Cyb-UE (B) and COI (C) were incubated with 20S editosomes for 60min to induce RNA unwinding. Unwound RNAs were visualized by AFM and outlines of 30-60 individual RNAs were manually traced using MFP-3D (Asylum Research, USA) to measure their contour length. The resulting contour length histograms were fitted to a Gaussian distribution. (D) Theoretical and measured contour lengths for the three different mRNAs.

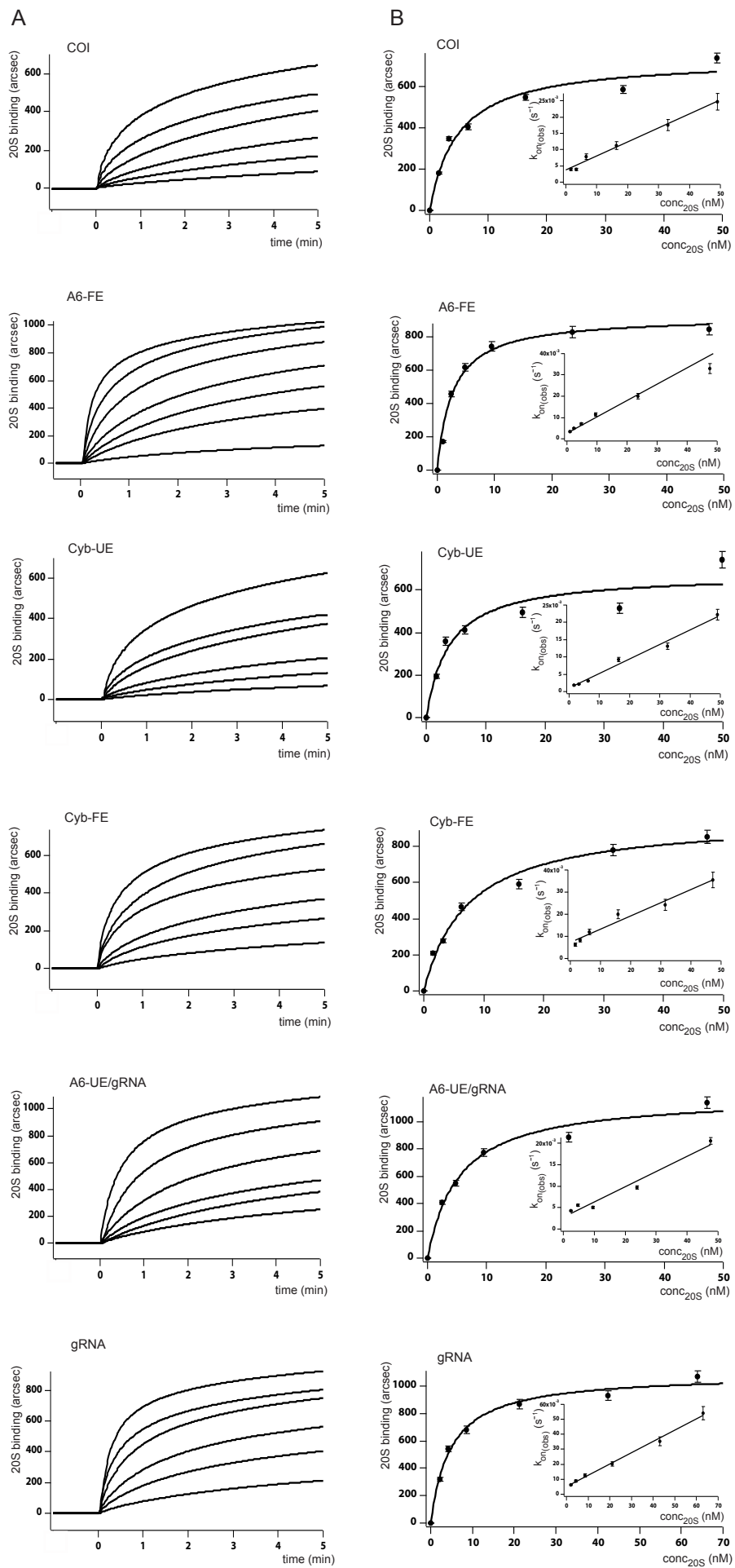


Fig. S1  
Böhm et al., 2012

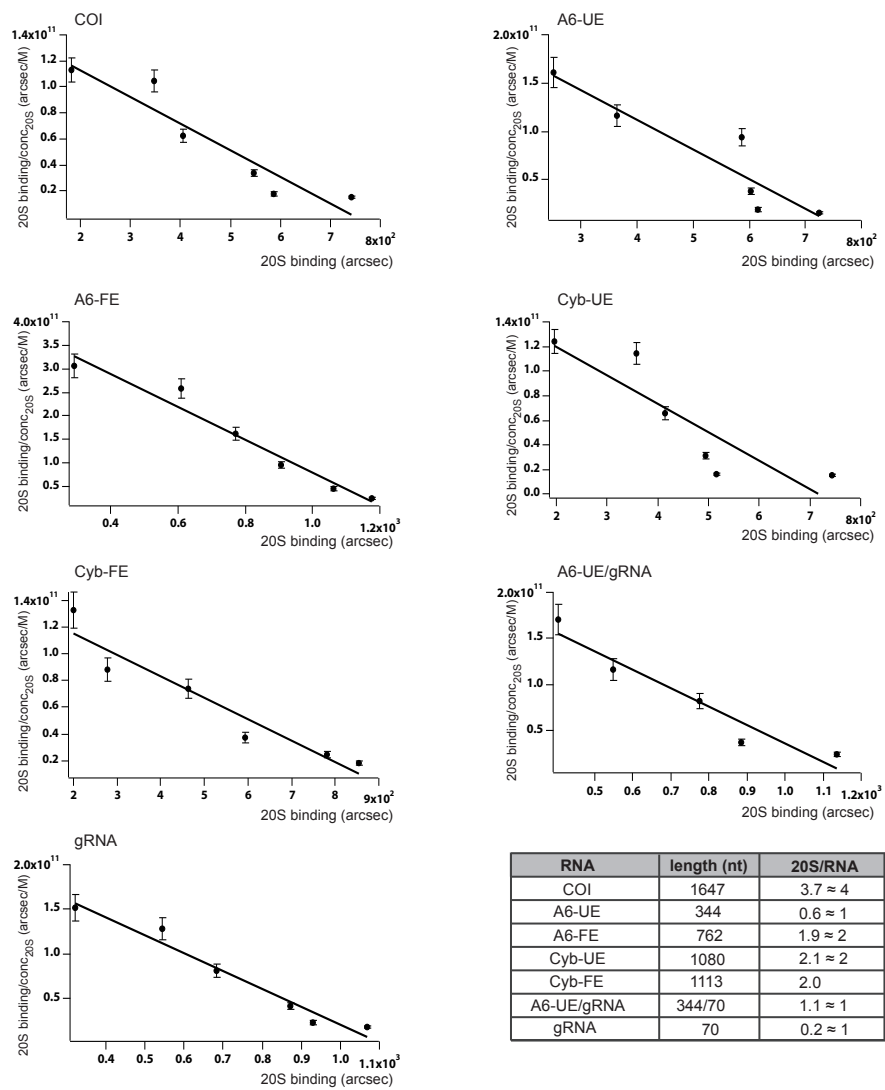


Fig. S2  
Böhm et al., 2012

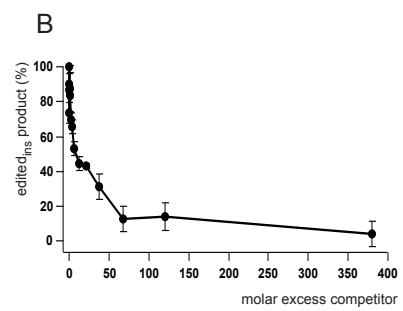
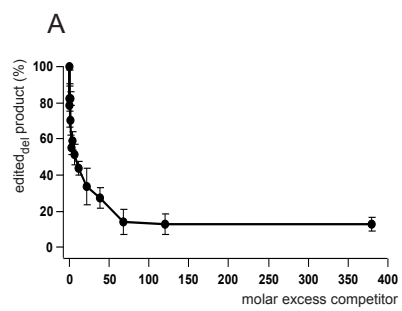
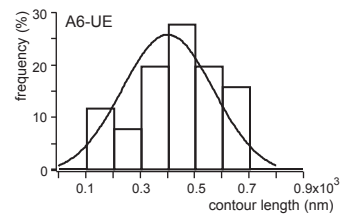
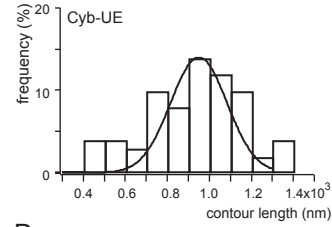


Fig. S3  
 Böhm et al., 2012

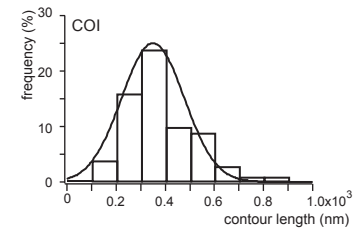
A



B



C



D

RNA	length (nt)	contour length theoretical (nm)	contour length measured (nm)
COI	1647	972	≈ 341
A6-UE	344	203	≈ 431
Cyb-UE	1080	637	≈ 956